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POSTLARVAL GROWTH IN JUVENILE *RHITHROPANOPEUS HARRISII*

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Abstract

Eyestalk removal accelerated the molt cycles of megalopal and juvenile (first through fifth crab instars) *Rhithropanopeus harrisii.* Eyestalkless crabs also demonstrated a greater increase in size at each ecdysis. The growth rate of eyestalkless crabs was approximately twice the rate measured in control crabs. Epidermal cell density measurements showed that the cell density was the same in intermolt fifth instar control and eyestalkless crabs. The results demonstrate that growth in juvenile crabs is under the influence of eyestalk neurosecretory centers and that growth is a result of epidermal cell proliferation and not cell enlargement.

Introduction

The growth rate of crustaceans is a function of both the molting rate and the increase in size obtained at each molt. In adults, these aspects of growth are thought to be regulated by hormones (see Passano, 1960; Kleinholz and Keller, 1979; Skinner, 1983 for review). The molting rate may be controlled by molt-inhibiting hormone (MIH) which is secreted by neuro-endocrine cells in the eyestalk. The eyestalk may also contain a factor that restricts the uptake of water at ecdysis and, consequently, the expansion of the new cuticle. The accelerated molting rate and the greater incremental increase in size observed in eyestalkless animals is believed to be a consequence of the absence of these two factors.

The action of endocrine factors in crustacean larvae and postlarvae, however, is not clearly defined. In early studies it was found that eyestalk removal did not result in a more rapid molting rate until the third post-larval instar in *Callinectes sapidus* (Costlow, 1963) or the fourth post-larval instar in *Rhithropanopeus harrisii* (Costlow, 1966). However, recent work in which the larvae were observed several times a day, revealed that eyestalk removal did elicit a faster molting rate in *R. harrisii* larvae (Freeman and Costlow, 1980).

In the present study, the effect of eyestalk removal during larval stages on molting rate, incremental size increase, and epidermal cell density in early juvenile *R. harrisii* is examined.

Materials and Methods

Larval rearing

The larval development of *R. harrisii* consists of four zoeal instars and one megalopal instar. Zoeae were hatched and mass reared in 25‰ sea water maintained at 20–21°C. The water was changed and freshly hatched *Artemia* were added daily.

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Upon reaching the megalopal instar the larvae were maintained individually in compartmentalized plastic boxes.

Eyestalk removal

Fourth instar zoeae were placed on a small glass disc (4 cm diameter) in a volume of water that was sufficient to keep them moist (50–100 μl), but small enough to restrict their movement. An iris scapel was used to sever the eyestalk at the articulating membrane. The larva was returned to 25‰ sea water immediately after the operation. Sixty percent of the eyestalkless larvae lived to molt to the megalopal instar. Of the larvae that molted to the megalopal instar, twenty five percent (9 of 36) lived to the sixth crab instar. Thirty nine percent (14 of 36) of the control crabs lived to the sixth crab instar. No abnormal megalopae, or supernumerary larvae were observed in either the intact or eyestalkless crabs.

Determination of molting and growth rates

Intact (control) and eyestalkless animals were observed twice daily for indications of ecdysis (presence of shed exoskeletons), and/or for apolysis (retraction of the epidermis from the cuticle). Apolysis indicates the initiation of the premolt phase (D_0) of the molt cycle (Drach and Tchernigovtzeff, 1967). Apolysis was determined through microscopic observation of the integument in the leg, rostrum, antennules, and antennae. Due to the opacity of the cuticle in third through fifth crab instars, apolysis was not followed in these crabs. The incremental growth at each instar was determined by measuring the differences in carapace width (CW) between the shed exoskeletons of that instar and the previous instar. The number of crabs observed for each measurement is indicated in the figures. Analysis of variance was done using the F-test. Significant difference between means was done with the *t*-test.

Measurement of epidermal cell density

Cell density measurements were done on whole mounts of hepatic or branchial sections (see McLaughlin, 1980) of dorsal carapaces removed from both intact (control) or eyestalkless fifth instar crabs. The specimens were fixed in Bouin's fluid, stained by the Feulgen method and mounted *in toto*. Cell counts were made from photographs of the stained whole mounts and are reported in the Results section as # nuclei/100 μm^2. Differences in cell density in control and eyestalkless crabs was determined with the *t*-test after analysis of variance.

Results

Eyestalk ablation during the late zoeal period accelerated the molt cycles of subsequent megalopal and juvenile instars (Fig. 1). The period from ecdysis to premolt (stage D_0) in eyestalkless crabs was significantly shorter ($P < .05$) than those of intact animals. The duration of the molt cycle (ecdysis to ecdysis) was also significantly reduced in eyestalkless crabs. These findings suggest that the eyestalks of the juvenile *R. harrisii* contain a factor that inhibits molting. The degree to which eyestalk removal shortened the molt cycle, however, varied from instar to instar. The molt cycles of the megalopal instar and fourth and fifth instar crabs underwent a greater reduction in duration, compared to control crabs, than did the molt cycles of the first, second, or third instar crabs.

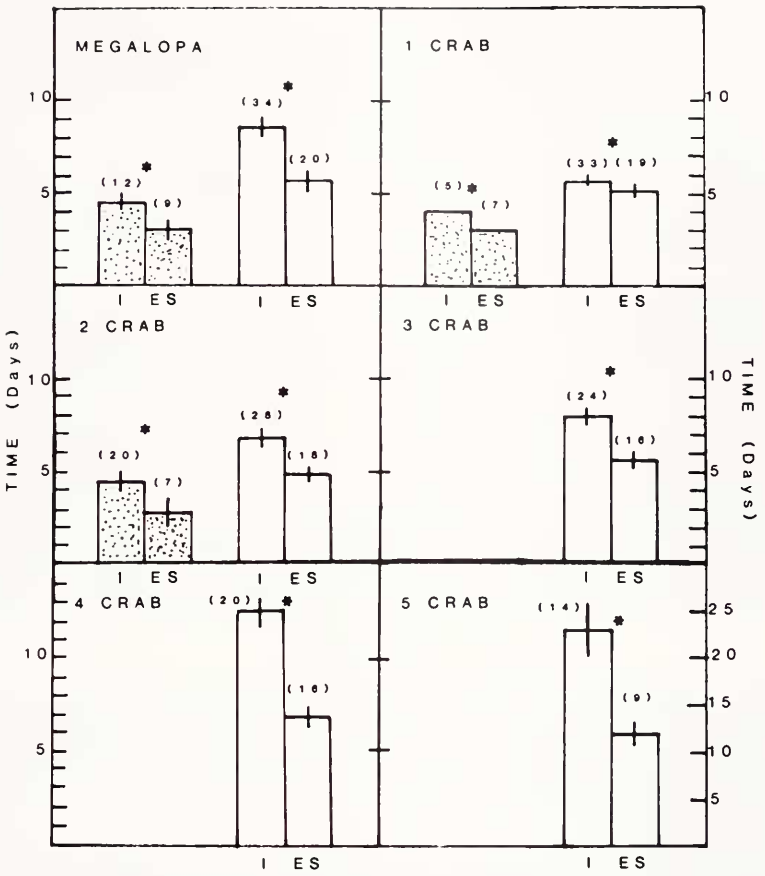

FIGURE 1. Duration (days) from ecdysis to premolt (D_0, stippled bars) and to the next ecdysis (open bars) in intact (I) and eyestalkless (ES) *Rhithropanopeus harrisii* megalopae and first through fifth crab (designated 1, 2, 3, 4, 5 crab, respectively) instars. Each bar represents mean ± 1 standard deviation. Bars without S.D. lines indicate no variation. Sample size for each measurement indicated in parentheses above the bar. Asterisk indicates significant differences ($P < .05$) between intact and eyestalkless groups.

Carapace widths of eyestalkless animals were always significantly larger ($P < .05$) than those of controls (Fig. 2). The actual difference in carapace widths between control and eyestalkless crabs was small during the megalopal and first two crab molt cycles. The differences increased, however, in the third, fourth, and fifth crab molt cycles. These data indicate that eyestalk removal affects the mechanism that regulates size increases at each ecdysis.

While it may be suspected that a crab would have more potential for growth if the molt cycle was longer, the results reported here show that just the opposite occurred in eyestalkless crabs. When data from Figures 1 and 2 are combined to yield a growth rate (mm carapace width/time, Table I) it can be seen that, even though the eyestalkless crabs reached the fifth crab in roughly two-thirds the time required by the control crabs, their growth was over twice that of control animals. To find if the growth rate varied in different instars, the increase in carapace width/instar was calculated (Table II). With the exception of the first crab, the eyestalkless

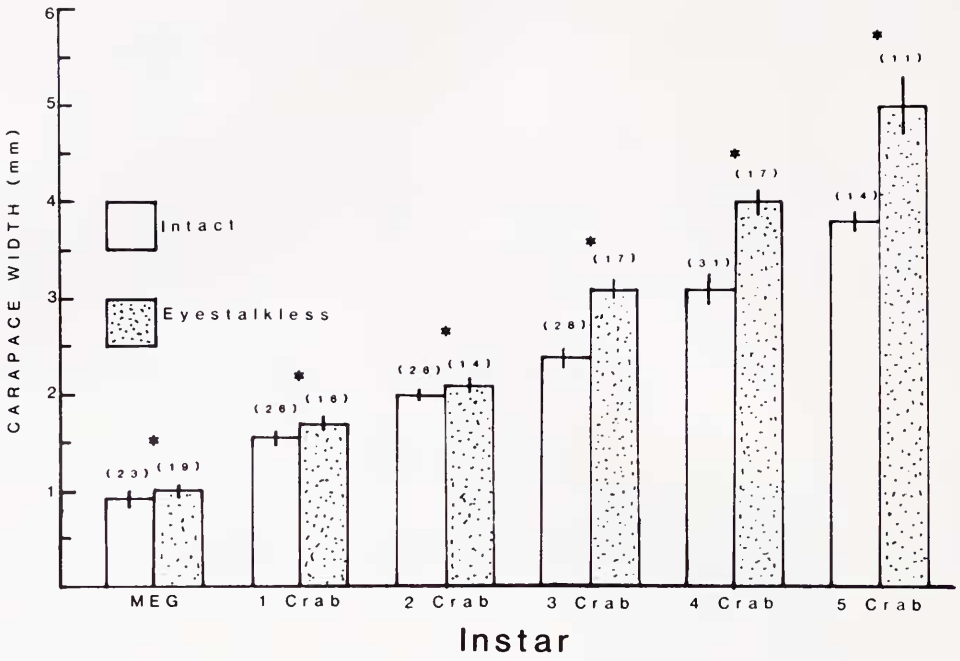

FIGURE 2. Carapace widths of intact and eyestalkless megalopae and first through fifth instar crabs (designated 1, 2, 3, 4, 5 crab, respectively) *R. harrisii*. Each bar represents mean ± 1 standard deviation. Width measurements were taken at end of instar by measuring shed exoskeleton. Sample size for each measurement indicated in parentheses above the bar. Asterisk indicates significant difference ($P < .05$) between intact and eyestalkless groups.

crabs demonstrated more growth per instar than did control crabs. Since the control and eyestalkless groups differed in molt cycle length at each molt cycle, the growth rates were calculated in terms of carapace width increase/day/instar (Table II). These calculations demonstrate that, in each of the molt cycles examined, there was a greater growth rate in the eyestalkless crabs.

If the greater incremental size increase of eyestalkless animals was strictly a function of excessive cuticular stretching caused by unrestricted intake of water at ecdysis, then the epidermal cell density of eyestalkless animals should be less than that of intact animals. When epidermal cell density determinations were made on

TABLE I

Growth in Rhithropanopeus harrisii *juveniles*

	Total time (days) from megalopa to fifth instar crab	Growth of carapace during period from megalopa to fifth instar crab (mm/day)
control	55.7*	.052**
eyestalkless	34.1	.117

* From Figure 1: Sum of mean durations for first-fourth crab molt cycles.

** From Figures 1 and 2: mean CW fifth crab minus mean CW megalopa/sum mean durations of first-fifth crab molt cycles.

TABLE II

Growth rates for juvenile Rhithropanopeus harrisii

Instar	Carapace width increase (mm/instar)*		Carapace width increase (mm/day/instar)**	
	Control	Eyestalkless	Control	Eyestalkless
Megalopa	.65	.70	.076	.125
First instar crab	.45	.45	.080	.089
Second instar crab	.40	1.00	.059	.204
Third instar crab	.70	.90	.090	.172
Fourth instar crab	.70	1.00	.056	.145

* From Figure 2, mean CW of instar n + 1 minus mean CW of instar n.
** From Figures 1 and 2, CW instar n + 1 − CW instar n/mean number of days per instar.

regions of the dorsal carapace, there was no significant difference in cell density between the control and eyestalkless crabs (Table III), even though the mean carapace widths of the two groups differed by nearly 25%. These findings suggest that the observed size differences between the control and eyestalkless crabs were not due to differences in cell size, but rather to enhanced cell proliferation in the eyestalkless crabs.

DISCUSSION

Our results show that eyestalkless juvenile crabs molt at a more rapid rate than intact crabs demonstrating that the eyestalks of juvenile crabs are involved in regulation of the molt and growth rates. This is in keeping with earlier findings (Freeman and Costlow, 1980) which showed that MIH is produced during the larval period.

The extent to which the molt cycle is accelerated in eyestalkless crabs varied during the megalopa and juvenile period. This suggests that the eyestalks may produce a molt-inhibiting hormone (MIH) in differing quantities during each of the zoeal, megalopal, and juvenile phases of the life cycle. It is possible that alterations in the molting frequency may be an adaptation to the different environments experienced by the three phases. Molt-inhibiting hormone is apparently present in reduced amounts during the zoeal period (Freeman and Costlow, 1980), thus allowing the larvae to grow and complete postembryonic development in the shortest period of time. While the plankton contains optimal amounts of food for zoeal growth, the longer the larva resides in the plankton, the greater the chance that it will be consumed by larger larvae or fish (see Morgan, 1981). Conversely, increased levels of MIH during the megalopal instar would lengthen the molt cycle, allowing the crab more time to take up a benthic existence and find a suitable habitat. Then,

TABLE III

Mean cell density of carapace from control and eyestalkless fifth instar crabs Rhithropanopeus harrisii

	Nuclei/100 μm^2
control	1.22 ± .27* (n = 16)
eyestalkless	1.10 ± .16 (n = 5)

* Mean ± 1 standard deviation.

during the early juvenile phase, minimal production of MIH would again permit rapid molting, providing a mechanism for rapid growth and onset of reproductive maturity, which occurs in the fifth crab instar (Payen *et al.*, 1969).

The results of the present study differ from those of earlier reports on molting in juvenile *Callinectes sapidus* (Costlow, 1963) and *R. harrisii* (Costlow, 1966). The discrepancy may be explained by two important differences in the experimental protocols. First, in this study, the animals were reared at 21°C while, in the earlier studies, the crabs were reared at 25°C. The lower temperature shows the molt cycle, thus making subtle differences between the intact and eyestalkless animals more evident. Second, both apolysis (stage D_0) and ecdysis were followed in the present study, while only ecdysis was noted in the earlier work. The observation schedule used here has been shown to be a more accurate means of assessing the rate at which an animal passes through the molt cycle stages (Freeman and Costlow, 1980).

Eyestalk removal also resulted in large increases in carapace width at each ecdysis, in keeping with earlier findings on larval crabs (Costlow, 1966) and shrimp (Little, 1969). Similar findings have been presented for adult *Uca pugilator* (Abramowitz and Abramowitz, 1940), *Cambarus* (Scudamore, 1947), *Carcinus* (Carlisle, 1955), *Homarus americanus* (Mauviot and Castell, 1976), and other crustaceans (see Passano, 1960). Enhanced growth in eyestalkless animals has been attributed to loss of a neurosecretory factor that regulates, in some manner, the rate of water influx at ecdysis (see Passano, 1960). Water uptake at ecdysis is a normal physiological event which serves to increase hemolymph hydrostatic pressure, thereby causing the rupture of the weakened old exoskeleton and unfolding of the epidermis from a plicated to a planar form (Drach, 1939; Passano, 1960). An abnormal increase in the influx could result in an actual stretching of the integument. Direct proof for a mechanism involving neurosecretory-controlled increase in water uptake, however, has not been forthcoming. Alternatively, as pointed out by Passano (1960), the increased extensibility may be due to a thinner exoskeleton at the time of ecdysis. This would occur if ecdysis took place earlier than normal during the premolt period when fewer lamellae would have been secreted in the new exoskeleton.

Initially, enhanced integumental stretch would result in each epidermal cell having an increased apical area. Findings obtained in this study show, however, that the epidermal cell density is the same in intermolt crabs from both the control and eyestalkless groups. For the cell density to be similar, while the growth rate was greater, there would have to be more cell proliferation in the integument of the eyestalkless crabs. It is, therefore, possible that the epidermis of eyestalkless animals responded to the stretch by increasing the amount of cell proliferation, thereby restoring the apical region of the epidermal cell to the normal area. At the present time, it is unclear if this enhancement of epidermal cell proliferation has an endocrine basis or if it is a result of the stimulation of metabolic processes that characteristically follow eyestalk removal (Kleinholz and Keller, 1979).

In the present study, we were able to maintain eyestalkless *R. harrisii* through one megalopal instar and five consecutive crab instars. In contrast, eyestalk removal in adult crabs often results in death after one molt. Seldom are several consecutive molts obtained. Although we can not explain this difference from the results reported here, it is possible that the relatively brief molt cycle duration of the juvenile *R. harrisii* (4–12 days) may allow them to molt several times before the detrimental effects of eyestalk loss become severe. Larger crabs often have molt cycle durations that are much longer than several days. In fact, the molt cycles of mature adult *R. harrisii* can last for two months (Freeman, unpublished observations), almost twice the total time for the larvae to pass through the first five crab instars (see Table I).

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